Data Evaluation Record

DIMETHOATE
PC Code 035001

EPA Contract No. EP10H001452
Task Assignment No. 1-31-2010
Study Type: Non-Guideline

Citation: Chen, H. et al. (2002) Estrogenicity of organophosphorous and pyrethroid pesticides. *Journal of Toxicology and Environmental Health, Part A.* 65**:** 1419-1435.

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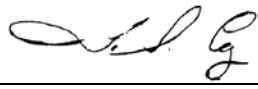
Signature: 
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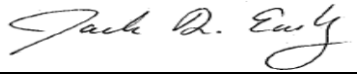
Signature: 
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DATA EVALUATION RECORD

STUDY TYPE: Non-guideline

DP BARCODE: D376890

PC CODE: 035001

CAS NO.: 60-51-5

MRID NO.: 48146201

TEST MATERIAL: Dimethoate (>90% purity)

CITATION: Chen, H.; Xiao, J.; Hu, G.; Zhou, J.; Xiao, H.; and Wang, X. (2002) Estrogenicity of organophosphorous and pyrethroid pesticides. *Journal of Toxicology and Environmental Health, Part A.* 65**:**1419-1435.

SPONSOR: This journal article was submitted as "Other Scientifically Relevant Information" (OSRI) in response to the Agency's Test Order for Tier 1 screening assay for the Endocrine Disruptor Screening Program. Test Order # EDSP–035001-38 and 39.

EXECUTIVE SUMMARY: The purpose of this study (48146201) was to evaluate the estrogenic activities of five organophosphorous (phoxim, malathion, monocrotophos, dimethoate, and optunal) and four pyrethroid (fenvalerate, permethrin, deltamethrin, and cypermethrin) insecticides, using *in vitro* methods: E-Screen assay, estrogen receptor (ER) competitive binding assay, and pS2 expression assay (pyrethroid pesticides only).

The E-Screen assay was developed to assess the estrogenicity of environmental chemicals using the proliferative effect of estrogens on their target cells as an endpoint. Human breast cancer estrogen-sensitive (MCF-7) cells, obtained from Shanghai Institute for Cell Science, Chinese Academy of Sciences, were grown/maintained in RPMI 1640 medium supplemented with 10% fetal bovine serum (FBS) at 37 ˚C in an atmosphere of 5% CO_2 and saturation humidity. Trypsinized cells were plated into 96-well plates with 5% charcoal-dextran stripped FBS in phenol red-free RPMI 1640 medium at initial concentrations of 10^3 cells per well. Cells were allowed to attach for 24 hours and then the seeding medium was removed and replaced with

medium containing a range of concentrations of the test compounds. The test compounds (>90% purity) were provided by the Environmental Endocrinology Laboratory of the Center for Bioenvironmental Research at Tulane school of Medicine, and prepared in either 100% ethanol or dimethyl sulfoxide (DMSO). 17β-estradiol (E_2; positive control) and dimethoate were tested in log increments at concentrations from 10^{-13} to 10^{-8} M, and 10^{-11} to 10^{-6} M, respectively. The bioassay was terminated on Day 6 by removing the medium and adding a thiazolyl blue solution (5 mg/mL phosphate buffered saline). After 4 hours, the solution was removed and 200 µL DMSO was added; after 10 minutes the absorbance was determined at 490 nm by an automated microplate reader.

Relative proliferation was determined by comparing the cell yield achieved by similar inocula harvested simultaneously during the late exponential phase of proliferation. The proliferative effect (PE) is measured as the ratio between the highest cell yield obtained with the test chemical and the hormone-free control and is expressed as X-fold proliferation. Estrogenic activity was assessed by relative proliferative potency (RPP) which is the ratio of maximum PE chemical to E_2. Proliferation yield experiments were conducted a minimum of six times.

For the ER competitive-binding assay, uterine cytosol was obtained in-house from adult female Sprague-Dawley rats using published methods. Following sacrifice, the collected uteri were homogenized at 4 ºC in TEDGSP buffer (10 mM Tris, 1 mM EDTA, 2 mM dithiothreitol, 10% glycerol, 10 mM sodium molybdate, 1 mM phenylmethylsulfonyl fluoride; pH 7.5), and centrifuged to yield cytosol. The cytosol was incubated with 10^{-9} M 2,3,6,7-[^{3}H]estradiol, TEDGSP buffer, and E2 (positive competitor control) at 10^{-11} to 10^{-4} M or dimethoate at 10^{-11} to 10^{-6} M. Following incubation at 4 ºC for 16 hours, cold dextran-charcoal buffer in TE buffer was added to separate the bound and free ligand. Radioactivity was measured using a liquid scintillation analyzer. Data for each competitor and the E_2 standard curve were plotted as percent [^{3}H]-E_2 bound versus molar concentration, and the IC_{50} (concentration of chemical necessary to inhibit binding of [^{3}H]-E_2 to the ER by 50%) for each competitor was determined.

Differences between groups were assessed by ANOVA with $p<0.05$ being regarded as significant.

Dimethoate showed no estrogenic potential in testing with the E-Screen assay. The maximum proliferative effect in the E-Screen assay was observed at 1 nM and higher for E_2 and under these conditions cell yields were 3-fold higher than those in the control (0.1% ethanol). Dimethoate had no significant effects on cell proliferation in comparison with vehicle controls at concentrations of 10^{-11} to 10^{-6} M. In the competitive binding assay, radioinert E_2 effectively competed with [^{3}H]-E_2 for binding to ER at the tested concentrations (10^{-11} to 10^{-4} M). Dimethoate did not inhibit binding of [^{3}H]-E_2 to ER at concentrations of 10^{-11} to 10^{-6} M.

The study was conducted by the Institute of Toxicology and Institute of Basic Medicine Sciences at the Nanjing Medical University (Nanjing, People's Republic of China). The study was reported according to the standards of the *Journal of Toxicology and Environmental Health*.

CLASSIFICATION: Not Applicable. This study does not conform to a recognized guideline requirement. The qualitative information provided in this study is considered to be acceptable for use during a weight-of-evidence evaluation of the OSRI.